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Changes in Plasma Amino Acid Concentrations in Response to HIV-1 Infection

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Plasma concentrations of 21 amino acids were determined for 20 control subjects and 20 subjects infected with human immunodeficiency virus type 1 (HIV). Compared with the control subjects, the HIV-infected group had lower cystine, tryptophan, and methionine (decreased 67%, 52%, and 32%, respectively, $P < 0.001$ for each) and increased taurine (230%, $P < 0.001$) and lysine concentrations (30%, $P < 0.001$). Other amino acid concentrations changed modestly. Amounts of cystine, tryptophan, methionine, taurine, and lysine did not differ significantly between subgroups of HIV-infected subjects with >200 ($n = 6$) or <200 ($n = 14$) $CD4^+$ lymphocytes per microliter, suggesting that the concentrations decrease soon after infection and change little thereafter. Activation of metabolism of cystine to taurine may explain reciprocal changes in these amino acids and known depletion of cystine and glutathione. The selective changes in amino acid profiles observed during HIV infection differ from those recognized for malnutrition or other pathological processes.

Indexing Terms: tryptophan/cystine/taurine/metabolism/nutrition

Infection with human immunodeficiency virus type 1 (HIV-1) triggers a range of metabolic changes in addition to the progressive deficits in cellular immunity and increased susceptibility to opportunistic infections that are its clinical hallmarks, and the progression to the acquired immunodeficiency syndrome (AIDS) (1, 2). The most tangible metabolic response to HIV infection is weight loss, due to a combination of factors including increased metabolic rate, anorexia, and malabsorption (3-10). Not infrequently, this weight loss progresses to a severe wasting syndrome (3, 8). There are also multiple, more subtle metabolic changes that can be measured: Early in HIV infection the resting metabolic rate increases (3-8), as do rates of protein turnover (3, 9). Concentrations of the adrenal steroid hormone dehydroepiandrosterone sulfate decrease (11), the anion gap increases (12), and disturbances in lipid metabolism produce decreased serum cholesterol and increased triglycerides (13). Plasma cystine and tryptophan (14-18) and intracellular glutathione concentrations also decrease (14-16).

The diverse metabolic responses to HIV infection may contribute to the pathophysiology of the disease. The

increased metabolic rate and protein turnover contribute to weight loss and muscle wasting. The decrease in cystine, which usually is the rate-limiting substrate for the synthesis of glutathione (14-16, 19), may be a factor in triggering the replication of HIV. Other findings indicate that intracellular thiols, such as glutathione, suppress HIV replication (20, 21). These results have led to *N*-acetylcysteine or other compounds that might boost glutathione concentrations being considered for therapy for HIV infection. Decreased availability of tryptophan may contribute to neuropsychiatric disease in HIV infection by decreasing production of the neurotransmitter serotonin, promoting affective disorders (17, 18). Also, tryptophan deprivation serves as a physiological defense mechanism against some intracellular parasites such as *Toxoplasma gondii* and *Chlamydia* species (22), and in this respect could be an adaptive response against infection.

The basis for the complex metabolic responses to HIV infection is not fully understood, but it is likely to be mediated by lymphokines. Concentrations of many lymphokines such as γ -interferon change dramatically after HIV infection (23, 24). γ -Interferon increases expression of indoleamine-2,3-dioxygenase, an enzyme that cleaves the indole ring of tryptophan (25-27). Induction of this enzyme by lymphokine responses to HIV infection may cause the decreased plasma concentrations of tryptophan (17, 18); there are concurrent increases in the concentrations of end products of tryptophan metabolism such as quinolinic acid (28, 29).

Although major changes in tryptophan and cystine concentrations are known to occur in HIV infection, there is limited information regarding other amino acids. Rather than a specific response to lymphokines, the decreases in cystine and tryptophan may represent a general depletion of essential amino acids analogous to that seen in starvation. Here we examined the concentrations of 21 plasma amino acids during HIV infection and compared their profiles in this disease with those during other pathological processes. An extensive database is available for comparison of amino acid profiles that have been characterized for many physiological and pathological conditions such as liver disease (30), starvation (31), pellagra (32), chronic pulmonary disease (33), posttraumatic injury (34), and various types of cancer (35, 36).

Materials and Methods

HIV infection of subjects was confirmed by testing for antibodies to HIV antigens and by confirmatory testing with Western blot analysis as part of standard protocols for enrollment in studies of the AIDS Clinical Trial Unit at Washington University. Protocols for enrollment of

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subjects and collection of specimens were approved by the Institutional Review Board at Washington University. Heparinized blood was collected from HIV-infected subjects during their routine visits for enumeration of CD4 lymphocytes. A portion of the whole-blood sample was used for flow cytometric analysis, and a portion was centrifuged for 15 min at 2000g to yield plasma for amino acid analysis. Samples were prepared for flow cytometry with the Q-Prep whole-blood lysis technique involving antibodies, lysis reagents, and equipment from Coulter Corp. (Hialeah, FL). We performed five analyses on each sample, using double antibody-labeling and a Profile I flow cytometer according to recently described guidelines for lymphocyte analysis (37). The proportion of CD4⁺ lymphocytes was determined by using reagents from Coulter with an antibody pair consisting of fluorescein isothiocyanate-conjugated antibody to CD3 and a phycoerythrin-conjugated antibody to CD4. Other antibody pairs were for gating markers, isotypic controls, CD8 counts, and B-cell counts. The total lymphocyte count was determined with a Coulter Model S counter by using a blood specimen collected concomitantly with other samples into a tube containing potassium EDTA. Plasma samples for amino acid analysis from HIV-infected subjects were selected so as to obtain a broad distribution of CD4 counts. Selection of samples was otherwise random, and samples represented a broad range of age, clinical status classified according to revised criteria of the Centers for Disease Control and Prevention (38), and treatment protocols as listed in Table 1. All but one sample were from males, despite active recruitment of female subjects. Control samples were collected from volunteers who by history were in good health and did not have unexplained weight loss. The age and gender distribution of the control subjects were similar to those of the experimental group. The control group had a mean age of 35 ± 10 years, vs 36 ± 10 for the experimental group.

Plasma samples for amino acid analysis were stored frozen at -20°C and prepared for analysis by addition of norleucine as an internal standard and of sulfosalicylic acid to bring the sample to a concentration of 6 g/L. After centrifugation, the pH of the supernatant was adjusted and 5- μL samples were analyzed on a Beckman 6300 analyzer (Beckman Instruments, Fullerton, CA) by postcolumn reaction with o-phthalaldehyde and fluorescence detection. Calibrators were run daily. The system does not detect secondary amino acids such as proline, hydroxyproline, and sarcosine. Values for aspartic acid are not reported, because they were below the limits of detection in many subjects. Concentrations of cystine and tryptophan in some subjects with HIV infection were below the lower limits of accurate quantification by the analyzer, which corresponded to a sample concentration of 6 $\mu\text{mol/L}$. In these analyses, values of 6 $\mu\text{mol/L}$ were assigned for these amino acids for the purpose of calculating group means and standard deviations. This applied to eight samples for cystine and two samples for tryptophan, all from HIV-infected subjects. The significance of differences between group means

Table 1. Characteristics of the HIV-infected subjects.

Age	Race	CD4 count	CDC stage*	Therapy
36	White	49	C3	AZT, DDI, Dapsone
29	Black	70	B3	DDI, TMP/SMX
28	White	476	B2	Blinded antiretroviral
20 ^b	Black	260	A2	Blinded antiretroviral
46	White	28	B3	AZT, pentamidine aerosol
36	White	15	B3	DDI, pentamidine aerosol
34	White	13	C3	AZT, TMP/SMX
48	White	195	A3	None
17	Black	166	B3	AZT, DDI, TMP/SMX
32	White	31	C3	DDI, pentamidine aerosol
48	White	314	A2	Blinded antiretroviral
33	Black	13	C3	AZT, TMP/SMX
50	White	188	A3	TMP/SMX
48	White	39	C3	AZT, α -interferon
38	White	451	A2	Blinded antiretroviral
30	Black	107	A3	AZT, DDI, pentamidine aerosol
31	White	301	A2	None
53	White	41	B3	AZT, DDC, TMP/SMX
40	White	27	C3	DDI, TMP/SMX
30	Black	296	A2	AZT

* CDC (Centers for Disease Control and Prevention) stage is defined by CD4 count: A, >500 ; B, 200–499; C, <200 ; and by symptoms: stage 1, asymptomatic or acute HIV infection; stage 2, symptomatic without AIDS-defining infections; stage 3, symptomatic with AIDS-defining illness, which include *Pneumocystis carinii* pneumonia, toxoplasmosis of the brain, *Mycobacterium avium* complex infection, extrapulmonary cryptococcosis or histoplasmosis, primary lymphoma of the brain, Kaposi sarcoma, and others listed in new criteria for AIDS (38).

^b Female patient. All other patients were male.

AZT, 3'-azidothymidine or zidovudine; DDI, 2',3'-dideoxyinosine or didanosine; DDC, 2',3'-dideoxycytosine; TMP/SMX, trimethoprim and sulfamethoxazole.

was assessed as a two-tailed probability with Student's *t*-test. Correlations of amino acid concentrations with CD4 counts were analyzed with InPlot software (Graph-Pad, San Diego, CA) and assessed with a two-tailed *t*-test. Considering that *t*-tests were performed for 20 sets of data corresponding to results for each of 20 different amino acids, there is ~ a 20-fold increased probability of noting a difference that had occurred by chance. For this reason, the Bonferroni correction (division of usual level of statistical significance, $P = 0.05$, by the number of comparisons) was applied and only differences with $P < 0.0025$ were considered statistically significant.

Results

The mean and standard deviations of plasma concentrations of 21 different amino acids in controls and subjects with HIV-1 infection are compared in Table 2. Results for glutamic acid and glutamine (Glu + Gln) were combined, because the samples were not processed immediately to prevent significant conversion of glutamine to glutamic acid. Mean concentrations of several essential amino acids were dramatically decreased in the HIV-infected group. Cystine decreased by 67%, tryptophan by 52%, and methionine by 32%. Other amino acids showed substantial increases, including taurine,

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Methion
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Tyrosine
Arginine
Threoni
Isoleuci
Leucine
Valine
Glu + C
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Table 2. Plasma amino acid concentrations.

		Mean \pm SD, $\mu\text{mol/L}$		
Amino acid		Controls (n = 20)	HIV-infected (n = 20)	Change, %
Cystine		46 \pm 20	15 \pm 13 ^a	-67
Tryptophan		46 \pm 22	22 \pm 12 ^a	-52
Methionine		28 \pm 8	19 \pm 6 ^a	-32
Asparagine		45 \pm 22	34 \pm 10	-24
Tyrosine		65 \pm 18	50 \pm 13	-23
Arginine		67 \pm 27	58 \pm 16	-13
Threonine		134 \pm 41	121 \pm 34	-10
Isoleucine		69 \pm 20	64 \pm 15	-7
Leucine		131 \pm 31	123 \pm 28	-6
Valine		216 \pm 33	208 \pm 43	-4
Glu + Gln		598 \pm 105	595 \pm 87	-1
Alanine		398 \pm 92	406 \pm 76	+2
Phenylalanine		56 \pm 16	57 \pm 14	+2
Histidine		71 \pm 6	74 \pm 15	+4
γ -Aminobutyric acid		15 \pm 10	17 \pm 10	+13
Serine		103 \pm 26	117 \pm 25	+14
Glycine		207 \pm 49	255 \pm 49	+23
Ornithine		94 \pm 22	121 \pm 38	+29
Lysine		173 \pm 35	225 \pm 37 ^a	+30
Taurine		47 \pm 11	155 \pm 63 ^a	+230

^a Statistically significant at $P < 0.001$.

which increased by 230%, and lysine, by 30%. All of these differences were significant ($P < 0.0025$), as indicated in Table 1. Amounts of most amino acids were not significantly changed. Concentrations of several essential amino acids such as leucine, isoleucine, valine, threonine, and phenylalanine were similar in experimental and control groups.

Comparison of taurine concentrations for the two subject groups (Fig. 1A) shows a marked increase in HIV-infected subjects, with almost complete discrimination between the two groups. There is overlap of only a single data point. Although there were substantial differences in the cystine (Fig. 1B) and tryptophan concentrations (Fig. 1C) between the two groups, there was greater variation among individuals and more overlap between HIV-infected and control subjects. Samples from control and HIV-infected groups were not obtained specifically from fasting subjects; this may increase experimental variability in the present study.

The relation between amino acid concentrations and the number of CD4⁺ lymphocytes during the course of HIV infection was examined. The depletion of CD4⁺ lymphocytes correlates with clinical progression of HIV infection, and a decrease in the CD4 count to < 200 per microliter has been added recently as a criterion for establishing the diagnosis of AIDS in patients with HIV infection (1, 37). Table 3 presents a comparison of amino acid concentrations in subgroups of HIV-infected individuals with CD4 counts < 200 or > 200 . No significant differences ($P < 0.0025$) were noted for any amino acids between the two subgroups, although asparagine, Glu + Gln, and phenylalanine were increased 29%, 19%, and 33%, respectively, and mean concentrations of cystine

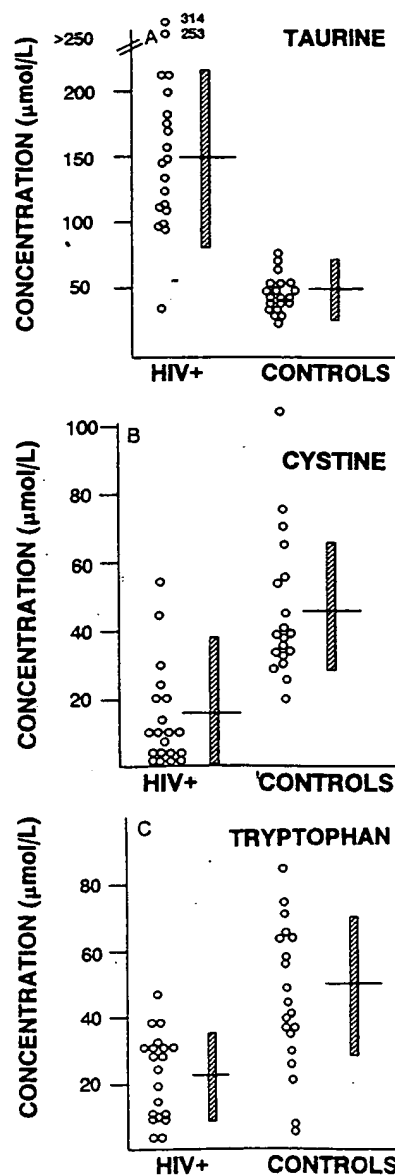


Fig. 1. Comparison of taurine (A), cystine (B), and tryptophan (C) concentrations in plasma of HIV-infected subjects and controls.

Bars adjacent to data points indicate mean and SD.

were 53% less and tryptophan 23% less in the low-CD4-count subgroup. Concentrations of leucine, lysine, isoleucine, valine, methionine, and threonine were unchanged or slightly increased in subjects with low CD4 counts. Comparison of the subgroups with a CD4 count $< \text{or} > 200$ indicated that progression of HIV infection to AIDS was not clearly associated with progressive depletion of any amino acid. In fact, amounts of some essential amino acids, such as phenylalanine, sometimes increased during the course of HIV infection. Amino acids in the eight subjects who were clinically asymptomatic (see Table 1) showed markedly different concentrations compared with the controls, similar to those of the total HIV-infected group. For the asymptomatic subgroup of HIV-infected subjects, the concentration of cystine was 19 ± 14 mmol/L (a 59% decrease vs controls), tryptophan was 22 ± 10 mmol/L (a 52% decrease), and taurine was 169 ± 68 (a 260% increase).

Table 3. Amino acid concentrations for HIV-infected subgroups.

Amino acid	Mean \pm SD, $\mu\text{mol/L}$	
	CD4 <200 (n = 14)	CD4 >200 (n = 6)
Cystine	12 \pm 12	20 \pm 16
Tryptophan	21 \pm 13	26 \pm 9
Methionine	20 \pm 7	17 \pm 2
Asparagine	36 \pm 4	28 \pm 4
Tyrosine	52 \pm 15	47 \pm 8
Arginine	62 \pm 17	50 \pm 11
Threonine	121 \pm 37	121 \pm 38
Isoleucine	65 \pm 17	60 \pm 10
Leucine	127 \pm 31	114 \pm 17
Valine	215 \pm 48	191 \pm 26
Glu + Gln	624 \pm 80	527 \pm 63
Alanine	423 \pm 91	365 \pm 91
Phenylalanine	61 \pm 14	46 \pm 5
Histidine	75 \pm 17	74 \pm 10
α -Aminobutyric acid	19 \pm 11	14 \pm 6
Serine	121 \pm 18	107 \pm 18
Glycine	265 \pm 46	232 \pm 46
Ornithine	126 \pm 33	109 \pm 49
Lysine	231 \pm 42	211 \pm 15
Taurine	148 \pm 55	172 \pm 81

No significant differences at $P < 0.0025$.

Changes in amino acid concentrations during the progression of HIV infection also were assessed by examining the correlation between amino acid concentrations and CD4 counts and treating the correlation as a continuous variable (plots not shown). At low CD4 counts, there were trends of increasing phenylalanine ($P < 0.02$) and Glu + Gln ($P < 0.03$), and trends of decreasing tryptophan and cystine (both $P > 0.05$). However, none of these relations reached the threshold of $P < 0.0025$ for significance of multiple comparisons.

Discussion

Individuals with HIV infection have diminished plasma concentrations of tryptophan and cystine (14-18). Data presented here indicate that depletions of these amino acids is highly specific. The only other amino acid observed to undergo a major decrease is methionine (though to a lesser extent than cystine), and loss of this amino acid may be explained by metabolic interconversion of cysteine and methionine (39).

HIV infection produced relatively little change in the concentrations of essential amino acids such as threonine, valine, isoleucine, leucine, and phenylalanine. In starvation, the concentrations of these amino acids as well as cystine and tryptophan are depleted relatively uniformly (31). Individuals with HIV infection have many factors contributing to malnutrition such as anorexia, intestinal malabsorption, intestinal parasitemia, and increased metabolic demands from infection (3-10). These factors contribute to decreases in the concentrations of many nutrients such as vitamins A, E, riboflavin, B₆, and B₁₂, and copper and zinc (40), but our

results indicate that general deficiencies of essential amino acids do not develop. The highly selective decrease of cystine and tryptophan in HIV-infected individuals indicates that these amino acids are not diminished because of a nutritional deficit. Specific pathways for the uptake or metabolism of cystine and tryptophan must be activated. A likely pathway for the consumption of tryptophan is via induction of the synthesis of indoleamine-2,3-dioxygenase (17, 18). Our finding of increased concentrations of taurine in HIV-infected subjects supports analogous consumption of cysteine via conversion to taurine, which can occur by two parallel pathways that are initiated by cysteine dioxygenase and cysteine decarboxylase, respectively. The pathway initiated by conversion of cysteine to cysteine sulfinic acid by cysteine dioxygenase is usually the predominant route, and activity of this enzyme is rate-limiting for the pathway (39, 41).

Previous work (17) and the data presented here provide evidence that decreases in plasma concentrations of tryptophan, cystine, and methionine occur relatively early in the course of HIV infection, well before development of clinically defined AIDS. These amino acids were already severely depleted in the subgroup of patients with CD4 counts >200; therefore there is a relatively weak trend of tryptophan and cystine relative to CD4 counts—most of the change had occurred earlier in the course of HIV infection. This is expected if the selective decreases in amino acid concentrations are mediated by changes in amounts of cytokines such as γ -interferon. Cytokine responses develop rapidly after HIV infection, even before development of antibody responses to HIV antigens (1, 23, 24). Not only were changes in amino acid concentrations not correlated with CD4 counts, but they also were not correlated with clinical staging of disease. Eight clinically asymptomatic subjects with HIV infection appeared to have marked changes in amino acid concentrations with respect to controls. Therapy of subjects with HIV infection is not considered a likely source of difference from controls because, as listed in Table 1, treatment of the HIV-infected subjects was with a variety of medications and two of the subjects received no medications.

The highly specific changes in the pattern of amino acid concentrations in HIV infection do not correspond to recognized patterns occurring in other pathological processes. Complete starvation or severe protein-calorie malnutrition results in a relatively symmetric reduction in essential amino acids (31). Many disorders such as sepsis, hepatic dysfunction, and advanced emphysema are characterized more by increases in selective amino acids than decreases (30-34). Studies of changes of amino acid concentrations in cancer patients note decreases of different amino acids with several types of malignancy (35). The reported pattern that is most similar to that in patients with HIV infection is that occurring in patients with lung cancer and breast cancer. However, individuals with these cancers appear to have lesser decreases in tryptophan and cystine than do individuals with HIV infection (36). More extensive anal-

essential amino acid patterns in HIV infection may be useful in characterizing the metabolic changes that occur early in infection. It remains to be established whether depletions of cystine and tryptophan are prognostic indicators or whether losses of these amino acids contribute to the pathophysiology of HIV infection. The highly specific responses of cystine and tryptophan metabolism could serve as second messengers or effectors for lymphokine responses.

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